STUDIES IN THE MOSAIC DISEASES OF PLANTS

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The particular group of diseases commonly known as "physiological" diseases has occupied the attention of botanists — pathologists and physiologists — for the last forty years, but it is doubtful whether the interest has ever been as keen as that which has been evidenced through the investigations and reports of the last few years.

Probably the commonest of the physiological diseases and the most studied, at least from the standpoint of the number of scientists whose attention it has occupied, is the one most generally known as the mosaic disease. The discovery of the disease, its appearance, and the results of the early investigators have been adequately reviewed in recent publications, while its occurrence on new hosts has furnished the subject matter of a number of short articles which have appeared recently. The object of this paper is primarily that of reporting the results obtained from experiments on mosaic diseases, but an attempt will also be made to review the observations and results of the early workers, with a view of interpreting them in the light of the results of recent investigations, and above all, to consider all evidence now known on the basis of the fundamental principles of physiology and pathology with the hope of arriving at a clearer conception of the cause and nature of mosaic diseases.

The most striking character is the differentiation of the green tissue of the blade into lighter diseased and darker apparently healthy areas. This naturally implies a difference in the chemical composition of the tissue and suggested a microchemical study of the differentiated areas of the diseased leaves. It was hoped that the chemical differences exhibited between sharply defined areas might furnish a clue to the cause of the anatomical and histological differentiations.

MICROCHEMICAL TESTS

In attempting the solution of a pathological problem by microchemical methods one must bear in mind that even individual healthy plants may, under apparently normal conditions, vary in their chemical composition. Differences in environmental factors, though relatively imperceptible, may be sufficient to change, for example, the acidity of plant tissues, while the more obvious effect of shade or decrease of illumination is evidenced in a decreased accumulation of fats, carbohydrates, and proteins. For this reason it is important that comparative analyses be made, not only on the same plant but on the same leaf and on adjacent areas. The microchemical methods now available are the results of investigations on normal healthy tissues, and some may think that their application to pathological tissues is unwarranted, and that the results obtained should therefore be interpreted with considerable reserve until the general application of the tests has been more definitely established. Microchemical tests, especially those outlined in the works of Molisch ('13) and Tunmann ('13), have arisen from innumerable tests on plants distributed throughout the vegetable kingdom. If their position is justified in the study of healthy tissue, they may well find a place in pathology.

The results obtained from a microchemical study should, whenever possible, be verified by macrochemical analyses or by other appropriate methods, as we are able to do for nitrogen by means of the Folin micro-Kjeldahl method. Considerable difficulty, however, will be experienced in getting enough material from well-differentiated areas of any one leaf or part of a leaf to make macrochemical analyses, and since no delicate macrochemical methods for the general analysis of plant tissues other than for nitrogen are available, the microchemical results reported below will have to suffice for the present. The results, furthermore, are not only relative as regards the comparison between different tissues, but are relative in themselves, since no absolute value can be determined and one is obliged to rely entirely upon impartial

judgment and the uniformity of results obtained from an extensive number of tests.

On account of the delicacy of the tests employed, all glassware and instruments used were cleaned with the greatest care. Even the pith used in sectioning the tissue was soaked and rinsed in alcohol and distilled water, and the sections were rinsed before the application of reagents, in order to insure the removal of all superficial and extraneous salts or foreign matter. The best reagents obtainable were used throughout the work. The results obtained with diseased tissue were always contrasted with those obtained with healthy tissue. The texts of Molisch ('13) and Tunmann ('13) not only served as an outline for the methods employed, but also as an index to the extensive literature on microchemical reactions.

NITROGEN

The detection of inorganic nitrogen in plants was first attempted in the work by Molisch ('83), and it is to these researches that all subsequent work owes its foundation. The test for nitrogen is based upon the fact that nitrates and nitrites give a blue color with diphenylamine, while a red color results upon the application of brucine.

Reaction with diphenylamine.—The most reliable of all tests for inorganic nitrogen is the one based on the reaction of nitrogen with diphenylamine. The reagent is applied in the form of .01–.1 gram of diphenylamine in 10 cc. reagent sulphuric acid. Since diphenylamine is insoluble, or only very slightly soluble, in water, it is necessary to apply the reagent to dry sections. When applied to wet sections, the diphenylamine will be precipitated and thus be unable to react with the nitrogenous compounds. Sections are therefore placed on the slide and allowed to dry, after which enough of the reagent is applied adequately to cover the mount. In the presence of nitrates a blue color results which gradually fades, but almost invariably shades into a light brown. The test may be negative or the reaction almost imperceptible in the presence of very minute quantities of nitrogen. In this

event, the reaction may be intensified by using thicker sections and a more concentrated solution of the reagent.

This reaction does not enable us to distinguish between nitrites and nitrates, but the former group of compounds has never been demonstrated in normal green tissues (Klein, '13) except in one plant, namely, *Erythrina coralloides* (Weehuizen, '09). The test employed by Weehuizen was as follows: The freshly expressed juice was tested with potassium iodide starch-paper. In the presence of nitrites a blue color developed which did not disappear upon treatment with sulphanilic acid and dilute sulphuric acid, but did turn to a carmine red upon the application of an alcoholic solution of alpha-naphthylamine. In adapting the test to the microchemical work, the potassium iodide starch-paper was made by soaking filter paper in a mixture of 50 cc. of $\frac{1}{4}$ per cent starch paste and 50 cc. of 3 per cent potassium iodide solution. A 0.1 per cent solution of alpha-naphthylamine was employed. Negative results were obtained when this test was applied to the juice expressed from diseased tobacco leaves.

Reaction with brucine.—Another test for inorganic nitrogen, though less delicate than that of diphenylamine, is the one with brucine. The reagent is applied as 0.2 gram brucine in 10 cc. reagent sulphuric acid. In the presence of nitrogen a deep red color is produced. In the presence of small quantities of nitrogen this test may fail completely, though results may have been obtained with diphenylamine.

When applying these tests to diseased tobacco tissue, fairly uniform results were obtained with diphenylamine. With brucine, however, the results were less satisfactory, being entirely negative or comparatively faint. This was true regardless of the kind of tissue examined, whether from the darker or the lighter areas. The results with diphenylamine, however, led the writer to conclude that not only is nitrogen present in both the lighter and darker areas, but that it is present in about the same quantity in both types of tissue.

AMMONIA

The presence of ammonia may best be determined by liberating the free gas by means of strong alkali, and collecting it with platinic chloride or Nessler's reagent. This can best be accomplished by placing a glass ring, the ends of which are smoothly ground, on a glass slide and placing in the center of the reservoir thus formed a drop of strong alkali. A drop of concentrated sodium hydroxide was used in the work reported here, and a narrow glass ring used for making hanging-drop cultures served to form the little compartment. The tissue to be tested was placed in the bottom of the compartment and enough sodium hydroxide was added adequately to cover the mount. After the addition of the alkali the compartment was covered immediately with a cover glass, to the lower surface of which there adhered a drop of platinic chloride or a drop of Nessler's reagent.

When Nessler's reagent was used, the drop assumed a deep yellow color which intensified, eventually resulting in the formation of a brown precipitate. In the case of platinic chloride, characteristic octahedral crystals of ammonium platinic chloride separated out. The detection of ammonia in plant tissue has been attempted on the part of various workers by applying Nessler's reagent directly to the sections. This test is, according to Molisch, unreliable, since various constituents of the tissue may not only change the color of the reagent to a yellow or brown, but a yellow color may be developed when alkali alone is applied to the tissue. Volatilization of the ammonia in the manner described is therefore the only reliable method for its detection.

When these tests were applied to the diseased leaves, splendid results of equal intensity were obtained regardless of the kind of tissue used. It was therefore concluded that salts of ammonia might, as a source of nutrition, serve all cells to the same degree.

TOTAL NITROGEN

An unbalanced nitrogen relation between diseased and healthy tissues has been cited as a partial explanation of the cause of mosaic diseases (Woods, '02). It therefore became

desirable to know whether the chlorotic area differed markedly in its nitrogen content from that of the adjacent green, and apparently healthy, tissue. In this work some of the older leaves had to be used, since the younger leaves, because of the limited amount of differentiated tissue, did not enable one to obtain enough material from adjacent areas to carry on the tests.

The Folin micro-Kjeldahl method was employed in this work. The results, however, especially for total nitrogen, were somewhat inconsistent, and the analyses can only be regarded as preliminary. The tests, nevertheless, showed no marked difference between the nitrogen content of diseased and healthy tissue. In fact, the nitrogen content of the lighter areas seemed to be slightly in excess of that of the darker areas. More extensive analyses are in progress at present, the results of which will be reported at a later date.

PROTEINS

The interesting results obtained from the nitrogen analyses suggested their possible correlation with the protein content of the differentiated areas. The limited amount of material made the extraction of protein impossible and microchemical tests were therefore resorted to. The tests commonly used in biochemical work were applied to both macerated tissue and hand-cut sections. Those used with advantage were the following:

1. Millon's test: Millon's reagent was applied to the material and the slide warmed gently over a micro-burner. A brick-red color developed, signifying the presence of protein. Millon's reagent consists of mercury dissolved in nitric acid (sp. gr. 1.42) in the proportion of 1:2 by weight. When the action of the acid on the mercury has ceased, the solution is diluted with water to twice its volume.

2. Biuret test: The material to be tested was placed on the slide and treated for about 15 minutes with a few drops of strong sodium hydroxide. The alkali was then allowed to drain off, and the material was rinsed with water and treated with a trace of 5 per cent copper sulphate. After several

minutes a violet color developed, indicating the presence of protein. This test is a comparatively difficult one.

3. Xanthoproteic reaction: Strong nitric acid was applied to the material and the slide warmed gently over a microburner. A yellow color developed which changed to orange upon the application of strong ammonia.

4. Iodine test: With iodine a deep yellow to brown color developed.

In all of these tests a more pronounced reaction was obtained with the lighter or diseased areas than with the darker. In the former the color showed up somewhat faster and was more intense. We may not be justified, however, in assuming that there is actually a great deal more protein in the chlorotic area than in the other, since the values in all of this work are only relative in themselves, and the excess of carbohydrates, etc. present in the deep green areas may obscure the above reactions in part. We would, however, be safe in stating that there is as much protein in the lighter areas as in the darker, and that there is a probability of there being more in the former than in the latter. The validity of this statement can only be determined by accurate quantitative methods.

It was originally intended to make analyses for amino nitrogen by the Van Slyke ('13) method, but because of the need of choice material for other work reported here, this determination had to be deferred.

IRON

Iron is one of the elements absolutely indispensable for plants, and may be present in the tissue in either organic or inorganic combination. Since it is universally conceded that a lack of iron is directly responsible for a certain type of chlorosis or the inability of the plant to form chlorophyll, it was desired to show, if possible, whether there was any marked difference between the iron content of lighter and darker areas of diseased leaves. In making these tests, iron-free chemicals, glass needles, and a new highly polished razor were used, thus obviating all possible sources of error.

Tests were made for ferric iron by treating the section on the slide for an hour or more with a 2 per cent solution of potassium ferrocyanide and then adding a 5 per cent solution of hydrochloric acid. In the presence of comparatively large amounts of iron, a deep blue (Berlin blue) color results. When only traces or minute quantities of iron are present, the reaction may be negative or a blue-green tinge developed. In this event it may be confused with the natural pigments and the results must be checked by more reliable methods.

Fairly uniform results were obtained with the method described, but further evidence was secured in the following manner: The surfaces of well-mottled leaves were washed and rinsed with distilled water, thus removing all foreign matter, and the well-defined areas cut out by means of a sharp glass needle. These were then dried in an oven. Lacking a platinum plate, the samples were ignited on a glass plate and the above reagents applied. A marked reaction resulted. This method, furthermore, possessed the desirable feature that no metal instruments were used in handling the material. No serious error, however, should have been introduced by cutting the sections with a highly polished razor.

The following test has been described for the detection of ferrous iron: The material is treated with a 2 per cent solution of potassium ferrocyanide or potassium cyanide for an hour or more. A few drops of 5 per cent hydrochloric acid are then added. In the presence of ferrous iron a blue color (Turnbull blue) results. This test proved negative in both diseased and healthy tissue.

When iron is present in organic combination it may be detected by incubating the material at about 60° C. with a solution of ammonium sulphydrate and 50 per cent glycerin mixed in aliquot proportions. The sections were placed on the slide, the reagent applied, and covered with a cover glass. Upon the liberation of the combined iron, varying from a few days to a few weeks, a very dark green or almost black color signified the presence of ferrous sulphide. Uniform results were not obtained with this method, but this might be attributed to the fact that the amount of iron present in the

small section was not sufficient to give a noticeable reaction.

From the tests on ferric iron it would appear that there is sufficient of this element present in all tissue to warrant the normal development of all cells.

CALCIUM

Calcium is generally detected as calcium sulphate. The sections were treated with a 3 per cent solution of sulphuric acid and allowed to stand until most of the solution had evaporated. Small plate-like crystals or needles of calcium sulphate were then noticeable in the remaining reagent, especially along the edges of the sections and in the intercellular air-spaces.

A second test applied was that with ammonium oxalate. The sections were treated with a 5 per cent solution of ammonium oxalate in a 10 per cent solution of acetic acid. The precipitate of calcium oxalate assumed the form of very small granules. The sections were tested further by adding a 5 per cent solution of oxalic acid containing a small amount of acetic acid. This gave satisfactory results, precipitating the calcium as minute crystals of calcium oxalate, pyramidal in form. The test was applied with equal success to all tissue.

MAGNESIUM

Besides being an indispensable element in the general nutrition of the plant, magnesium is important as an antidote for calcium and as a constituent of chlorophyll. Tests for magnesium were made by treating the sections with a 0.1 per cent solution of $NaH(NH_4)PO_4$ and placing the slide in a moist chamber containing a vessel filled with strong ammonia. The ammonia vapor killed the tissue and rendered the cell easily permeable to the sodium ammonium phosphate. After several minutes, crystals of magnesium ammonium phosphate separated out. These were either short and triangular in form, or, depending upon the quantity of magnesium present and also upon the time for which the reagent was allowed to react, were x-shaped or stellate in form, the appendages assuming a feather-like structure.

A more pronounced reaction was obtained by igniting bits of tissue and triturating the residue on the glass plate with a 10 per cent solution of hydrochloric acid. The liquid was then drained off, a large drop of $NaH(NH_4)PO_4$ applied, and the slide allowed to remain for several minutes in an atmosphere of ammonia.

Positive results were obtained when these tests were applied to both lighter and darker areas.

POTASSIUM

The most reliable test for potassium is its reaction with platinic chloride, resulting in the formation of crystals of potassium chloroplatinate. A 10 per cent solution of platinic chloride is recommended for this test. The sections to be tested were mounted in a drop of alcohol, and a drop of platinic chloride about one-tenth the size of the drop of alcohol was placed on the slide. The reagent and alcohol mount were then brought into communication by means of a glass needle. After several minutes crystals of potassium chloroplatinate, mainly in the form of octahedrons, but also in the form of hexahedrons and rhombohedrons, separated out.

This result was checked by applying a test solution consisting of 2 grams of cobalt nitrite and 3.5 grams of sodium nitrite dissolved in 1 cc. of acetic acid diluted with water to 7.5 cc. After the cessation of the liberation of nitric oxide fumes, the solution was diluted to a volume of 10 cc. Upon the application of this reagent to sections, minute granules of potassium cobalt nitrite separated out. The crystals were extremely small and were detected with difficulty.

These tests were applied with equal success to both diseased and healthy tissue.

PHOSPHORUS

Phosphorus is generally detected by means of a solution of 1 gram of ammonium molybdate in 12 cc. nitric acid (sp. gr. 1.18). In the presence of phosphorus, granules or small octahedrons of ammonium phosphomolybdate separate out.

A second test described for the detection of phosphorus is its precipitation as ammonium magnesium phosphate. The solution used for the determination consists of 25 volumes of a saturated aqueous solution of magnesium sulphate, 2 volumes of a saturated aqueous solution of ammonium chloride, and 15 volumes of water. This solution when applied to salts of phosphorus yields crystals of ammonium magnesium phosphate such as were described above under magnesium.

The writer was unable to get uniform results with either of these tests, regardless of the kind of tissue to which they were applied. When samples of diseased and healthy tissue were ignited a faint indication of the presence of crystals was detected at intervals in the residue, but the results on the whole were not encouraging. Because of the fact that this was experienced with healthy and normal tissue to the same degree as with diseased tissue, we may be justified in concluding that the disorder cannot be attributed to a marked unbalanced phosphorus relation.

SULPHUR

Sulphur is absolutely essential for plant growth, usually being present in organic combination. When present in inorganic form it may be detected as calcium sulphate by means of calcium acetate, or as lead sulphate with lead acetate, or as barium sulphate with barium chloride. Organic sulphur can best be detected after its liberation by ignition of the tissue.

The tests for sulphur gave results much the same as those for phosphorus. It was difficult to demonstrate its presence even in normal healthy tissue, and we are therefore unable to correlate any metabolic disturbance with a lack or a superabundance of this element.

TESTS FOR CARBOHYDRATES

From the marked difference in chlorophyll content of the lighter and darker areas of diseased leaves, it seemed self-evident that there must be a great difference in the carbo-

hydrate content. Tests were therefore made for starch and sugar as described below.

The material to be examined was cut into thin sections, consisting entirely of either lighter or darker tissue, or, in order to make the comparison more striking, in part of dark tissue and in part of light tissue.

Starch.—Tests for starch were made by mounting the section in water and drawing the slide through the flame of a micro-burner until the drop of water began to simmer. This not only killed the cells, but also expelled the air from the intercellular spaces, thus making observation easier. A drop of 75 per cent alcohol and a drop of standard iodine were then added, after which the section was examined under the microscope and the amount of starch noted. In general, it may be stated that whenever cells of the same section composed of different leaf areas, or cells of the same section representing adjacent differentiated areas were compared, there was an excess of starch in the dark green tissue. When testing for starch in the manner described, one cannot misinterpret the observations. It would be aside the point to offer the criticism that the difference in starch content may be attributed to the location of the tissue tested in different parts of the plant, to a probable shading of one tissue and not the other, to a difference in the age and therefore a difference in the storage and in the photosynthetic activity, or to other environmental factors. Any difference exhibited in cells of the same section representing different areas must be due to factors inherent in the tissue. This excess of starch in the green tissue was noticed regardless of the time of day when tests were made.

Woods ('02) cites an experiment which led him to conclude that starch was not translocated readily from the chlorotic areas, and attributed this fact to the inhibitory action exerted on diastase by oxidizing enzymes. The leaves were picked early in the morning and tested for starch by immersing in boiling water for one minute, decolorizing with alcohol, treating with iodine solution, and examining by transmitted light. A darker color, presumably, was assumed by

the chlorotic spots. It was also observed that when unboiled juice from tobacco leaves, possessing strong oxidase activity, was added to a digestion mixture of starch and taka-diastase, no diastatic action took place. When the juice was boiled and the oxidizing enzyme was killed before addition to the digestion mixture, diastatic action occurred. Combining these observations, Woods concluded that the inability of the chlorotic spots to rid themselves of their starch, as seemed to be demonstrated by his tests, was attributable to the action of oxidases on diastase.

In order to test this idea of Woods more conclusively, the writer examined diseased leaves early in the morning by the method described above, but always found an excess of starch in that portion of the section representing the darker area. Plants were then placed in the dark room and kept there for 54 hours. At the end of this time sections were cut and examinations made for starch. Observations on a number of leaves and a large number of sections showed that at the end of this period no starch whatever was present in the lighter or chlorotic areas, while the mesophyll of the darker areas still contained varying amounts. This, then, is in accord with the general observation that the dark tissue contains more starch regardless of the time of day when tests are made. It is quite probable that oxidizing enzymes may influence the activity of diastase, but when adding a strong extract from diseased tobacco leaves to a starch digestion mixture, we are adding innumerable unknown factors, the effects of which might easily be confused with those exhibited to a more or less pronounced degree by one of the known constituents when added alone in a pure state.

Sugar.—A microchemical test for sugar which has been long employed is the production of a fine red precipitate when treated with dilute Fehling's solution. Sections of tissue were placed on the slide, a drop of Fehling's solution added, and the mount warmed gently over a flame. No satisfactory results were obtained, which, in part at least, might be attributed to the great diffusibility of the sugar out of the cells as soon as they were killed and the difficulty with which the

small quantity may be detected in the test solution bathing the section.

A more reliable method is the one based on the reactions of sugars with phenylhydrazine to form osazones. This method has been developed most satisfactorily by Manghan ('15). Although the method may be criticized as to the reliability of the anticipated reaction of any one sugar in the presence of other sugars and as to the justification of attributing certain results to the reaction of the reagent with any one carbohydrate when other sugars are present, it nevertheless serves as a pretty fair qualitative test. Separate solutions of phenylhydrazine hydrochloride and sodium acetate were prepared by dissolving these reagents in sufficient pure sugar-free glycerin to make a 10 per cent solution. Small drops of these solutions were placed on the slide, mixed with a glass needle, and in this the sections to be tested were immersed. The mount was then covered with a cover glass and the preparation heated for an hour at 100° C. At the end of this period and continuing for several days, yellow bodies, resembling the droplets of syrup described by Manghan for maltose, and the small yellow spheres and granules figured by Molisch and Tunmann, separated out. The reaction was more pronounced in the darker areas of diseased leaves than in the lighter tissue.

From the results reported above it is very evident that one great difference between the dark green and chlorotic areas of diseased tissue is a difference in the carbohydrate content, there being more in the former than in the latter.

It was originally intended to accompany the above descriptions with adequate illustrations and to make similar tests on all varieties of plants showing mosaic. Microchemical methods, however, are very difficult, requiring the finest technique and the greatest care in observation and interpretation. Since, therefore, a general microchemical study of plants affected with mosaic is in reality a problem in itself, and also because of the nature of the results reported above, the microchemical work was deferred for the time being for

a line of experimentation which seemed more fundamental. The tests described above were carried out on diseased and healthy tobacco plants. The results are primarily relative, yet the relativity is to a certain degree quantitative, and enough so to justify us in concluding that calcium and nitrogen may be more abundant in the chlorotic areas, while carbohydrates are more plentiful in the green tissue. The cause of this unbalanced condition is not apparent at present. It is essential, however, that all these observations be substantiated by reliable quantitative analyses.

Physiological Relations

From the results reported above, and especially those relating to the inorganic constituents of the tissue, it is very evident that the real cause of the disease is more deeply seated than has been intimated in some literature on mosaic diseases. There is therefore reason to believe that the cause, if not associated with parasites, is essentially organic in nature, as is suggested by the work of Woods ('02) and others.

Woods ('02) maintained that mosaic diseases could be attributed to an excess of oxidases, since a more pronounced oxidase reaction was evidenced by diseased plants, and also when ridding his extract of oxidases, he lost the infective mosaic principle. However, this may not be the right interpretation, and to the writer this increase in oxidase reaction seemed to be an effect and not a cause of the disease. We would not conclude that the diminished sugar content of the lighter areas of the tissue as compared with that in the darker areas is responsible for the disease, but rather that this, as also the difference in oxidase activity, is in reality only the result of the disorder.

The writer therefore undertook to eliminate the oxidases from extracts of diseased plants without destroying their infectious properties, or, if it were possible, to secure a solution which from the start possessed infectious properties but no oxidase activity. Since Allard ('14, '15) has shown that the infective principle may be obtained from all parts of the plant

including the root system, and since it has been repeatedly stated in the literature that plants grown on land which had borne diseased plants were sure to contract the disease, an attempt was made to secure a root secretion possessing infectious properties. Roots were washed free from soil and then suspended in sterile distilled water. This method had to be abandoned, however, on account of bacterial growth.

Since the disease is most pronounced in growing tissue, i. e., in young shoots, and since the infective principle may be isolated from the roots as well as from all parts of the plant, it must be granted that the infectious substance is transferable from one plant part to another. This implies that some of the infectious substance, originally in a highly diseased shoot or any which might be formed subsequently, might be transferred to other plant parts. The question then arose: If this assumption is correct, would it be possible to secure by "shoot secretion" or by "shoot exudation" a solution possessing infectious properties? Furthermore, would any oxidases be present in this secretion?

An attempt was made to solve the question in the following manner: A large but badly diseased shoot of tobacco was removed from an old plant and after sterilizing the base with alcohol and rinsing with sterile distilled water, it was supported, through a rubber stopper, in a wide-mouthed bottle containing sterile distilled water. A piece of glass tubing inserted into a small hole in the rubber stopper served as an inlet for sterile water stored in a separatory funnel. All joints were closed tightly with paraffin and wax. The bottle containing the base of the shoot was filled with water, leaving no air whatever in the chamber. As the water was removed by transpiration it was replenished from the separatory funnel serving as a reservoir. This funnel was stoppered with a one-holed rubber stopper into which a piece of glass tubing stuffed with cotton was inserted.

The system was allowed to run from April 1 to April 19, 1916, at the end of which time the shoot had transpired 485 cc. of sterile water. Inoculations were made on April 19, 1916, with the secretion, and checks were run with juice ex-

pressed from diseased leaves and filtered through Berkefeld filters, and with sterile water. Four plants were used in each case. At the end of 17 days, 3 plants inoculated with the secretion were diseased, while all 4 of the plants inoculated with the filtered juice of diseased leaves showed mosaic. The 4 checks remained healthy.

This experiment was repeated on July 7, 1916, an entire plant being used for secretion. This necessitated the use of larger vessels, etc., which increased chances for contamination. The secretion obtained in this case had been badly fermented by bacteria, and inoculation experiments gave negative results.

The writer's time then became occupied with a field experiment and a repetition of the above had to be deferred. It was repeated, however, on September 6, 1916, the secretion phase of the experiment being allowed to run until October 19. Inoculations were made on October 22, 6 plants being used. After 15 days 2 of the 6 plants inoculated with the secretion showed mosaic, while all 6 inoculated with sterile tap water were healthy. Inoculations were not made with juice of diseased leaves as in the first experiment. There is reason to believe, from the uniformity of the results obtained with secretions, that some of the infectious substance was dissolved in the sterile water used for subsequent inoculations. Each secretion was tested for oxidases by adding hydrogen peroxide and guaiacum, as did Woods, but no oxidases were detected. No indisputable conclusions could be drawn from these results, since these experiments were only preliminary, but because of their uniformity, they furnished considerable encouragement towards investigating further the possibility of securing an extract possessing infectious properties but no oxidase activity.

On *a priori* grounds it seemed scarcely possible that substances such as oxidases found naturally and so commonly in plants should, when injected into other plants, be able to produce such a disorder as the mosaic disease. It will be recalled that Allard ('15[a]) was able to produce the disease even at a dilution of 1:10,000. One can easily see, however, how a sub-

stance naturally "toxic" to a plant might, even in extreme dilution, cause serious metabolic disturbances. During the time that the above work was deferred on account of another phase of the problem, an article was published by Allard ('16[a]) on some of the properties of the infective principle which showed conclusively that the mosaic disease of tobacco is not caused by oxidases but, according to Allard's interpretation, is caused by an ultramicroscopic parasite. The writer's preliminary work substantiates the more elaborate experiments of Allard, thus eliminating oxidases as a cause of the disease. I do not, however, concur with him in the interpretation of the properties of the infective principle, as will be noted later on.

PLOT EXPERIMENTS

A great deal of interest has been aroused recently by the mosaic diseases of cucurbits, particularly of cucumber. During the spring of 1916 cucumbers grown in the experimental greenhouse contracted the disease, and an experiment was planned to test its transmissibility not only to other cucurbits but also to other plants susceptible to the malady. All seeds were started in the greenhouse and later transplanted to the plot.

Injury to seedling at the time of transplanting has often been cited as predisposing plants to the disease, and it therefore became desirable to eliminate this factor. Tobacco seed was sown in flats and after 10 days the seedlings were transplanted to small paper boxes. The cotyledons had just expanded at the time of transplanting, and the entire plant measured about $\frac{1}{2}$–$\frac{3}{4}$ inches in length. Although exceedingly small, the seedlings could be handled without injury other than probably the destruction of a few root hairs. The paper boxes to which the plants were transferred were $2\frac{1}{2}$ inches long, $1\frac{1}{4}$ inches wide, and about 2 inches deep. When the plants were large enough to be transplanted to the plot, the entire box was submerged in the soil. This eliminated all possible chances for injury. Tomato seedlings were obtained by planting the seed in flats and handling the seedlings in the

manner described for tobacco, or the seed was sown directly in the paper boxes, as was the case with the cucurbits. Later in the season seeds were also planted directly in the soil. During the growing season the plants were cultivated with the greatest care in order to avoid injury. All plants which were damaged were either discarded or labeled so that any inconsistency in results might receive its proper explanation. The cucurbits grown included 2 varieties of pumpkins, 2 of squash, 2 of watermelon, 2 of cucumber, and 1 variety of citron, muskmelon, and cassaba. At the end of 2 months, when the plants had developed several runners, inoculations were made with an extract from diseased cucumbers. In cases where runners were numerous, 3 or 4 inoculations were made on the same plant, the total on all varieties summing up to 213. Not a single infection resulted from these inoculations.

Before offering an explanation it will be necessary to give some details regarding the preparation of the extract. For this purpose 500 grams of highly diseased shoots were gathered from diseased cucumber plants. The material was first washed in tap water and then rinsed in distilled water. Maceration was effected by placing portions of this material in a large mortar and pounding, crushing, and grinding the leaves and stems until all had been reduced to a pulp. The juice was then expressed through a cloth, and the residue washed with water until the original extract and the washings totaled 500 cc. An attempt was then made to filter off the substances suspended in the extract; but due to exceedingly slow filtration, other means had to be resorted to. An asbestos mat was therefore deposited in a Buchner funnel, which in turn was connected with a filter flask and filter pump. The filter, however, soon became clogged and filtration was not effected with the desired rapidity. The entire extract, asbestos and all, was therefore placed in centrifuge tubes and centrifugated until all the suspended material had been deposited. The supernatant liquid, which was of a slightly greenish color, was used for inoculations. About fifteen hours

elapsed between the time that the material was gathered and the time when inoculations were made.

As has already been stated, no infections resulted from these inoculations, and the experiment is of absolutely no value as far as throwing further light upon the transmissibility of cucumber mosaic to other cucurbits; but it does afford the writer an opportunity to criticize the technique involved, to point out probable sources of error, and to lay special emphasis upon the amount of care which must be exercised when dealing with something, the nature of which is so incompletely known.

If the infective principle is of the nature of an organism, one can easily see how the parasite might have been destroyed mechanically during the maceration of the tissue. Fred ('16), for example, has been able to reduce the number of bacteria in 1 gram of dry soil from 2,000,000 to 400,000 by grinding for 1 hour. In another instance the number was reduced from 3,194,000 to 75 by grinding for 24 hours.

Allard ('16[a]) found that the "virus" was extremely sensitive to the antiseptic properties of formaldehyde. Warner ('14) has demonstrated that formaldehyde is one of the oxidation products of chlorophyll extracts. The loss of the infectious properties of the cucumber extract used for inoculating might, in the absence of any proof whatever, be accounted for in this manner.

If the infective principle is of the nature of an enzyme or colloidal substance it is also possible, as with bacteria, that destruction took place through mechanical agitation. It is a well-known fact that many colloids, especially suspensoids, are thrown out of the colloidal equilibrium by mechanical agitation. This fact was furthermore demonstrated by Brown ('15) working on the macerating and lethal enzymes of *Botrytis cinerea*. He found that this action could be reduced eight-ninths by simply bubbling air through the extract for 45 minutes. Allard ('16[a]) also found that the "virus" was greatly adsorbed by talc. Whether or not the adsorptive power of the asbestos used, coupled with centrifugation, can account for the removal of the infective principle from the

extract is of course unknown, but this is another possibility.

Although no infections could be attributed to inoculations with the cucumber extract, the disease was, during the season, contracted by plants of pumpkin, squash, citron, cassaba, and two varieties of cucumbers. Some observations in this connection are also reported by Doolittle ('16).

Temperature and Moisture Relations

Although it is occasionally stated in the literature on mosaic diseases that high temperature favors the disease, there are, nevertheless, no quantitative data nor detailed experiments to substantiate this view. An interesting observation in this connection was made during the summer of 1916. Tomato plants which had been transplanted to the plot grew normally until the latter part of July when, due to drought and high temperature, they discontinued growing but otherwise appeared perfectly normal. These climatic conditions continued until the middle of August when several rains occurred which were followed, though not immediately, by comparatively cool weather. It was during these 10 days of cool weather, from August 23 to about September 2, that mosaic began to show on the tomato plants. During this period growth was resumed and all new shoots and buds were noticeably affected.

Following this comparatively cold period, there was another hot spell during which there not only was a high departure from the normal but the maximum for the individual days was higher, reaching 94° F. During this second, though relatively short, warm period accompanied by reduced precipitation growth ceased and the mosaic began to disappear. The chlorotic areas became darker, and the small amount of expansion which did take place in the leaves of the new shoots was not accompanied by malformations. Had it not been for the fact that the plants were under constant observation, the periodic occurrence of mosaic would have escaped notice entirely. No records pertaining to temperature and moisture were taken on the plot, but the following tables from the U. S. Department of Agriculture Weather Report present the

monthly meteorological summaries for St. Louis. Although these values are not absolute as regards weather conditions on the plot, they are sufficiently accurate to indicate the climatic differences during these time periods.

TABLE I

METEOROLOGICAL SUMMARY FOR JULY, 1916

DAY	TEMPERATURE (Degrees Fahrenheit)				MOISTURE			SUNSHINE		CHARACTER OF DAY
	Highest	Lowest	Mean	Departure from normal	Relative humidity percentage 7 a. m.	Relative humidity percentage 7 p. m.	Total precipitation (midt. to midt., inches)	Number of hours	Percentage of possible	
1	94	78	86	+ 8	65	53	.00	13.0	87	Clear
2	96	78	87	+ 9	66	46	.00	14.8	100	Clear
3	94	72	83	+ 5	64	65	.04	9.8	66	Pt. cloudy
4	86	69	78	0	76	52	.00	13.0	88	Clear
5	86	70	78	0	57	44	.00	14.8	100	Clear
6	87	69	78	0	58	42	.00	14.8	100	Clear
7	89	70	80	+ 1	67	52	.00	14.1	95	Clear
8	91	76	84	+ 5	73	40	.00	14.5	98	Clear
9	87	68	78	— 1	79	50	.00	14.7	99	Clear
10	88	66	77	— 2	74	42	.00	14.8	100	Clear
11	92	73	82	+ 3	68	47	.00	14.7	100	Clear
12	92	72	82	+ 3	61	70	.34	5.8	39	Pt. cloudy
13	95	72	84	+ 5	86	48	.00	14.7	100	Clear
14	92	77	84	+ 5	73	67	.00	9.9	67	Clear
15	94	77	86	+ 7	79	44	.00	14.3	98	Clear
16	97	80	88	+ 9	62	59	.01	11.1	76	Clear
17	96	79	88	+ 9	74	54	.00	6.8	47	Pt. cloudy
18	94	76	85	+ 6	78	55	T.	8.4	58	Pt. cloudy
19	94	70	82	+ 3	78	74	.81	9.2	63	Pt. cloudy
20	92	74	83	+ 4	83	68	.00	12.4	86	Clear
21	90	75	82	+ 2	87	41	.00	12.5	86	Clear
22	90	74	82	+ 2	59	35	.00	14.5	100	Clear
23	94	75	84	+ 4	48	48	.00	14.0	97	Clear
24	97	79	88	+ 8	55	52	.00	14.4	100	Clear
25	97	78	88	+ 8	63	44	T.	11.1	77	Clear
26	98	78	88	+ 8	62	45	.00	12.5	87	Clear
27	97	81	89	+ 9	65	41	.00	14.1	99	Clear
28	98	79	88	+ 8	68	52	.00	14.3	100	Clear
29	96	80	88	+ 8	75	44	.00	14.3	100	Clear
30	99	82	90	+11	73	46	.00	11.4	80	Pt. cloudy
31	99	79	89	+10	74	67	T.	12.1	85	Pt. cloudy

It is of course impossible to say whether this phenomenon should be attributed entirely to temperature relations or whether it was also determined, in part at least, by water relations. Many writers have reported that moisture seems to

favor the disease and that infection seems to be worse in plants growing in moist, clayey soil. There are, however, no quantitative data on the water requirements of healthy and diseased plants. On studying the weather chart, one can

TABLE II
METEOROLOGICAL SUMMARY FOR AUGUST, 1916

DAY	TEMPERATURE (Degrees Fahrenheit)				MOISTURE			SUNSHINE		CHARACTER OF DAY
	Highest	Lowest	Mean	Departure from normal	Relative humidity percentage 7 a. m.	Relative humidity percentage 7 p. m.	Total precipitation (midt. to midt., inches)	Number of hours	Percentage of possible	
1	89	72	80	+ 1	75	62	.00	11.1	78	Clear
2	90	71	80	+ 1	83	71	.34	7.0	49	Pt. cloudy
3	94	73	84	+ 5	87	47	.00	12.8	91	Clear
4	96	77	86	+ 7	81	56	.00	13.4	95	Clear
5	94	78	86	+ 7	81	58	.00	14.0	100	Clear
6	93	77	85	+ 6	79	48	.00	12.9	92	Clear
7	94	72	83	+ 4	70	57	.27	9.7	69	Pt. cloudy
8	87	73	80	+ 1	89	69	.36	6.8	49	Pt. cloudy
9	90	73	82	+ 3	96	77	.00	12.9	93	Clear
10	93	76	84	+ 5	87	64	T.	11.0	79	Clear
11	94	70	82	+ 4	79	62	.94	11.6	83	Pt. cloudy
12	91	69	80	+ 2	95	72	2.10	9.3	67	Pt. cloudy
13	73	67	70	— 8	98	75	.99	1.2	9	Cloudy
14	74	59	66	—12	100	91	3.67	2.3	17	Cloudy
15	85	67	76	— 2	100	74	1.73	7.8	57	Pt. cloudy
16	85	71	78	0	95	76	.00	9.2	67	Pt. cloudy
17	94	75	84	+ 7	89	67	.00	13.5	99	Clear
18	95	78	86	+ 9	78	56	.00	11.7	86	Clear
19	94	80	87	+10	72	60	.00	11.0	81	Pt. cloudy
20	96	80	88	+11	72	57	.00	11.9	88	Clear
21	94	77	86	+10	70	59	.00	10.1	75	Clear
22	85	66	76	0	77	63	T.	5.8	43	Pt. cloudy
23	80	61	70	— 6	74	48	.00	13.4	100	Clear
24	85	66	76	0	67	44	.00	13.4	100	Clear
25	88	68	78	+ 2	68	55	.00	13.3	100	Clear
26	86	65	76	+ 1	85	70	.20	5.4	41	Pt. cloudy
27	71	61	66	— 9	87	75	.09	0.8	6	Cloudy
28	75	55	65	—10	74	48	.00	10.1	77	Pt. cloudy
29	78	63	70	— 5	65	57	.00	11.1	84	Pt. cloudy
30	83	65	74	0	68	56	.00	11.9	91	Clear
31	84	66	75	+ 1	71	55	.00	10.6	81	Pt. cloudy

easily see how the above observation might also be explained on the basis of moisture relations, but this is not substantiated by the experiment next described.

On January 29, 1917, 6 badly diseased tomato plants were

placed in each of 2 separate compartments in the experimental greenhouse, one of which was kept at 75–95° F. (average 85° F.), while the other was held at a temperature of 35–50° F. (average about 45°).[1] After a period of 7 to 10

TABLE III

METEOROLOGICAL SUMMARY FOR SEPTEMBER, 1916

DAY	TEMPERATURE (Degrees Fahrenheit)				MOISTURE			SUNSHINE		CHARACTER OF DAY
	Highest	Lowest	Mean	Departure from normal	Relative humidity percentage 7 a. m.	Relative humidity percentage 7 p. m.	Total precipitation (midt. to midt., inches)	Number of hours	Percentage of possible	
1	74	66	70	— 4	83	95	.37	0.2	2	Cloudy
2	83	65	74	0	99	73	.00	7.4	57	Clear
3	85	67	76	+ 2	94	70	.00	11.0	85	Clear
4	90	69	80	+ 7	88	59	.00	8.6	67	Pt. cloudy
5	94	73	84	+11	82	55	.00	12.9	100	Clear
6	93	71	82	+ 9	78	52	.00	12.8	100	Clear
7	91	66	78	+ 5	71	86	.89	7.5	59	Pt. cloudy
8	78	67	72	0	90	63	.00	9.8	77	Pt. cloudy
9	80	63	72	0	80	62	.00	11.2	88	Clear
10	84	64	74	+ 2	82	60	.00	10.9	86	Clear
11	85	70	78	+ 7	86	76	.00	9.8	78	Pt. cloudy
12	79	66	72	+ 1	87	79	.10	3.7	29	Cloudy
13	70	63	66	— 5	75	66	.00	5.0	40	Cloudy
14	77	55	66	— 5	92	70	.00	12.5	100	Clear
15	64	49	56	—14	71	56	.00	12.5	100	Clear
16	72	49	60	—10	71	55	.00	12.0	97	Clear
17	67	53	60	—10	77	70	T.	11.4	92	Clear
18	64	48	56	—13	61	52	.00	12.3	100	Clear
19	72	52	62	— 7	53	49	.00	12.3	100	Clear
20	79	54	66	— 3	63	58	.22	4.3	35	Cloudy
21	76	61	68	0	62	52	.00	9.8	80	Clear
22	73	56	64	— 4	55	65	.00	12.2	100	Clear
23	71	51	61	— 7	73	53	.00	12.1	100	Clear
24	77	56	66	— 1	72	62	.00	7.3	60	Pt. cloudy
25	86	61	74	+ 7	84	47	.00	12.0	100	Clear
26	86	67	76	+ 9	81	51	.00	10.5	88	Clear
27	83	65	74	+ 8	69	100	1.11	6.4	53	Cloudy
28	65	46	56	—10	85	70	T.	0.4	3	Cloudy
29	59	41	50	—16	77	54	.00	11.9	100	Clear
30	63	45	54	—12	63	60	.00	11.8	100	Clear

days the plants in the room kept at high temperature showed less mottling, while 40 days later, at the time of this writing,

[1] The temperature values are given in terms of Fahrenheit in order to facilitate comparison with the temperatures recorded in the meteorological summaries.

no mottling whatever can be detected. The plants in the compartment kept at 45° F., at this time, still show a great deal of mottling. The writer has not had time to carry on inoculation experiments. Ten tobacco plants were also placed in each

TABLE IV
METEOROLOGICAL SUMMARY FOR OCTOBER, 1916

DAY	TEMPERATURE (Degrees Fahrenheit)				MOISTURE			SUNSHINE		CHARACTER OF DAY
	Highest	Lowest	Mean	Departure from normal	Relative humidity percentage 7 a. m.	Relative humidity percentage 7 p. m.	Total precipitation (midt. to midt., inches)	Number of hours	Percentage of possible	
1	68	45	56	— 9	72	49	.00	11.8	100	Clear
2	74	50	62	— 3	80	52	.00	11.8	100	Clear
3	79	54	66	+ 1	70	56	.00	11.7	100	Clear
4	81	57	69	+ 5	61	49	.00	11.7	100	Clear
5	84	62	73	+ 9	59	44	.00	9.8	84	Clear
6	82	60	71	+ 8	91	55	.00	11.6	100	Clear
7	86	65	76	+13	78	57	.00	11.5	100	Clear
8	84	67	76	+14	79	64	.00	5.5	48	Pt. cloudy
9	72	48	60	— 2	88	71	T.	0.0	0	Cloudy
10	59	42	50	—12	85	51	.00	11.4	100	Clear
11	65	46	56	— 5	63	46	.00	8.5	75	Pt. cloudy
12	78	54	66	+ 5	54	61	T.	6.1	54	Cloudy
13	69	55	62	+ 2	76	42	.00	9.2	81	Pt. cloudy
14	68	48	58	— 2	69	49	.07	6.4	57	Pt. cloudy
15	59	52	56	— 3	100	93	.22	0.0	0	Cloudy
16	75	55	65	+ 6	94	75	.01	4.1	37	Cloudy
17	60	46	53	— 5	71	48	.00	11.1	100	Clear
18	53	45	49	— 9	80	100	.33	0.0	0	Cloudy
19	58	38	48	— 9	95	100	.27	0.0	0	Cloudy
20	39	31	35	—22	94	84	.05	0.0	0	Cloudy
21	51	30	40	—16	78	58	.00	11.0	100	Clear
22	65	38	52	— 4	70	60	.00	10.5	96	Clear
23	68	45	56	+ 1	69	54	.00	9.3	85	Clear
24	73	53	63	+ 9	64	53	.00	4.1	38	Pt. cloudy
25	62	45	54	0	86	58	.01	6.0	56	Pt. cloudy
26	64	40	52	— 1	85	40	.00	8.2	76	Clear
27	71	52	62	+ 9	54	47	.00	10.7	100	Clear
28	75	54	64	+12	65	38	.00	6.8	64	Pt. cloudy
29	75	54	64	+12	61	51	T.	4.0	38	Pt. cloudy
30	74	50	62	+11	94	74	.68	8.8	83	Clear
31	64	50	57	+ 7	82	76	.00	10.6	100	Clear

compartment, but the results with tobacco were somewhat different, since the plants differ physiologically from tomatoes.

The tobacco plants kept at 85° F. remained diseased, while those kept at 45° showed at least temporary recovery. This

is illustrated in pl. 14. Plant No. 2 was kept at 85° F. and shows the young leaves passing through a venation stage into true mottling. Plant No. 1 kept at 45° F. shows the reverse. Mottled leaves pass through the venation stage into an ap-

TABLE V

METEOROLOGICAL SUMMARY FOR NOVEMBER, 1916

DAY	TEMPERATURE (Degrees Fahrenheit)				MOISTURE			SUNSHINE		CHARACTER OF DAY
	Highest	Lowest	Mean	Departure from normal	Relative humidity percentage 7 a. m.	Relative humidity percentage 7 p. m.	Total precipitation (midt. to midt., inches)	Number of hours	Percentage of possible	
1	71	47	59	+ 9	58	42	.00	10.5	100	Clear
2	67	51	59	+10	58	26	.00	10.5	100	Clear
3	66	48	57	+ 8	46	36	.00	1.2	12	Pt. cloudy
4	79	60	70	+22	66	58	.00	7.4	71	Clear
5	79	59	69	+21	76	74	.00	10.4	100	Clear
6	76	56	66	+19	79	44	.00	10.4	100	Clear
7	75	59	67	+20	71	52	.00	10.3	100	Clear
8	71	53	62	+16	76	59	.63	1.4	14	Cloudy
9	56	44	50	+4	91	29	.10	7.4	73	Clear
10	67	44	56	+11	64	47	.00	10.2	100	Clear
11	62	41	52	+7	90	56	.00	10.2	100	Clear
12	57	39	48	+4	89	78	.00	6.5	64	Cloudy
13	39	22	30	—14	93	76	.05	0.0	0	Cloudy
14	29	15	22	—21	75	54	.00	10.1	100	Clear
15	34	20	27	—16	72	41	.00	10.0	100	Clear
16	53	25	39	— 4	72	43	.00	10.0	100	Clear
17	48	31	40	— 2	78	46	.00	6.3	63	Pt. cloudy
18	51	28	40	— 2	86	39	.00	10.0	100	Clear
19	68	42	55	+13	62	34	.00	9.9	100	Clear
20	68	44	56	+14	56	41	.00	9.9	100	Clear
21	49	36	42	+ 1	91	86	T.	0.0	0	Cloudy
22	55	46	50	+ 9	77	93	1.29	0.0	0	Cloudy
23	53	38	46	+ 6	89	70	.46	1.2	12	Cloudy
24	39	30	34	— 6	66	45	.00	9.8	100	Clear
25	43	26	34	— 6	70	44	.00	8.4	86	Pt. cloudy
26	56	35	46	+ 6	55	37	.00	9.8	100	Clear
27	58	42	50	+11	42	47	.00	6.0	62	Pt. cloudy
28	60	50	55	+16	88	79	.00	1.3	13	Cloudy
29	56	46	51	+12	91	41	.00	9.7	100	Clear
30	55	36	46	+ 7	66	28	.00	9.6	100	Clear

parently healthy stage, while the young leaves show but a slight venation and are nearly healthy. It was impossible to keep the temperature of this room down to 45° F. during and after the early part of March, and it occasionally reached

84° F. The plants which showed apparent recovery at the low temperature grew vigorously and showed some mottling.

A single potato plant which had contracted the disease was discovered in the greenhouse, and this was placed in the room

TABLE VI

METEOROLOGICAL SUMMARY FOR DECEMBER, 1916

DAY	TEMPERATURE (Degrees Fahrenheit)				MOISTURE			SUNSHINE		CHARACTER OF DAY
	Highest	Lowest	Mean	Departure from normal	Relative humidity percentage 7 a. m.	Relative humidity percentage 7 p. m.	Total precipitation (midt. to midt., inches)	Number of hours	Percentage of possible	
1	58	40	49	+10	55	44	.00	9.6	100	Clear
2	60	41	50	+12	62	52	T.	8.0	83	Clear
3	68	49	58	+20	78	74	.00	7.0	73	Pt. cloudy
4	71	58	64	+26	81	53	T.	6.6	69	Pt. cloudy
5	59	45	52	+14	61	24	.00	6.8	71	Clear
6	57	39	48	+10	41	32	.00	7.1	74	Clear
7	69	50	60	+23	81	92	.37	0.0	0	Cloudy
8	58	29	44	+ 7	89	79	.57	0.0	0	Cloudy
9	38	24	31	— 6	72	56	.00	9.5	100	Clear
10	38	28	33	— 3	82	55	T.	5.8	61	Pt. cloudy
11	36	24	30	— 6	83	89	.02	0.0	0	Cloudy
12	30	17	24	—12	84	82	T.	1.0	11	Cloudy
13	20	7	14	—22	71	76	.00	3.1	33	Cloudy
14	19	7	13	—23	83	83	.07	0.0	0	Cloudy
15	26	2	14	—22	87	56	T.	6.2	66	Pt. cloudy
16	52	16	34	— 1	72	61	.00	9.4	100	Clear
17	38	27	32	— 3	92	63	T.	0.0	0	Cloudy
18	30	19	24	—11	66	64	T.	6.0	64	Clear
19	40	23	32	— 3	71	80	.00	0.4	4	Cloudy
20	23	10	16	—19	94	68	.02	1.9	20	Cloudy
21	12	8	10	—24	84	88	.04	0.0	0	Cloudy
22	21	2	12	—22	79	64	.00	9.4	100	Clear
23	36	18	27	— 7	85	78	.01	6.5	69	Cloudy
24	47	30	38	+ 4	77	60	T.	7.1	76	Pt. cloudy
25	38	26	32	— 2	82	69	.00	9.4	100	Clear
26	58	37	48	+14	98	98	1.06	0.0	0	Cloudy
27	46	29	38	+ 5	82	73	.00	6.1	65	Pt. cloudy
28	35	26	30	— 3	69	52	.00	9.4	100	Clear
29	31	22	26	— 7	63	55	.00	9.4	100	Clear
30	34	22	28	— 5	66	59	.00	9.5	100	Clear
31	40	24	32	0	69	59	T.	6.9	73	Pt. cloudy

kept at 85° F. At the end of 10 days nearly all mottling had disappeared, and the plant was then placed in the room kept at 45°. At the time of transfer, the sprouts which had been trailing on the ground were tied up. The leaves gradually

began to drop off, presumably due to excessive transpiration, until only a few remained at the top. These later became chlorotic, but they were not uniformly colored, being speckled with green areas. Whether or not this can be interpreted as a recurrence of the disease or merely an unmasking of the formerly diseased areas cannot be stated positively, but it probably is the latter. The plant died shortly after this last observation was made. The case of this potato plant is, of course, but a single instance, but it is entirely in accord with the other observations.

No record was kept of the amount of water supplied to the plants in the various experiments. They were all watered according to their normal needs under greenhouse conditions. The greenhouse observations substantiate those on the plot. When the tomatoes on the plot showed no mosaic, the tobacco and some of the cucurbits were greatly mottled.

It therefore not only seems that individual plants exhibit an optimum for the mosaic in accordance with the optimum of their growth, but that there also may be a maximum beyond which little or no mosaic is manifested. If this is true we should also find a minimum, and some very interesting observations have been made in this direction. In a recent article, Brierley ('16) reports recovery of tomato plants from mosaic. Inoculations were made by him, but he states that "unfortunately the plant was killed outright by frost ten days later, at that time showing no sign of disease." From this we would conclude that the plant was kept in a comparatively cool place. This same phenomenon was observed in tobacco plants kept at 45° F., as has been stated above and shown in pl. 14.

Another observation was made in the fall of 1916. Plate 15, fig. 1, shows two plants (*b* and *c*) which are the same age, the one perfectly healthy, the other presumably affected with mosaic. On November 20 plant *b* was removed to a greenhouse to which no heat was supplied. The photograph of plant *a* was taken on December 13, 1916, after which plant *b* was placed in a greenhouse maintained at 65–75° F. in order to note a probable recurrence of the disease. The plant, however, remained healthy and fruited normally. No

greenhouse records are available for the cool greenhouse, but since no heat was supplied, the temperatures were in the neighborhood of those recorded in the monthly meteorological summaries given above.

The question, of course, arises as to whether plant *b* is really affected with mosaic. The leaves are not truly mottled, but are venated. The malformation is characteristic, particularly of some of the new shoots appearing on old diseased tobacco plants. When discussing the effect of temperature on the plants shown in pl. 14, it was stated that the leaves passed through the venation stage to true mottling or apparent recovery. It therefore seems that the venation stage is a transitional stage of mosaic, and as far as external appearances go, it is in this stage that we find plant *b* in pl. 15, fig. 1. The plants shown in pl. 14 assumed a healthy appearance by the gradual darkening of all the spaces between the veins, while in the case of diseased leaves only some of the veins "run together," the area between others remaining chlorotic and the leaf becoming truly mottled. The histology of this has not been worked out satisfactorily, but "recovery" in strongly venated leaves seems to be effected by a lengthening of the palisade cells and a normal elongation and growth of the cells in general. In the chlorotic areas the palisade cells remain short, division is infrequent, and elongation of the cells in general is retarded. The relation of growth to the development of the disease is of great importance.

Another interesting observation on temperature relations is the following: Plate 15, fig. 2, shows a plant, the lowest shoot of which (*c*) is slightly mottled. The other shoots at the base (*a* and *b*) show no mottling whatever. Shoots *a* and *b* appeared during the time when the temperature of the greenhouse was low and sunlight was not abundant. Shoot *c* appeared during the early part of January when the temperature was higher and the illumination better. The plant was taken into the laboratory on January 10 to be photographed, and on account of the inclemency of the weather was allowed to remain there until January 13, when it was observed that some of the large leaves were becoming

chlorotic. The plant was therefore removed to the greenhouse in spite of the danger of freezing. On January 16, it was noticed that the plant had been severely chilled and that the edges of nearly all the leaves were turning black. The three shoots referred to above were removed, macerated in 5 cc. of distilled water, and the extract used for inoculations. The shoots weighed from 1.5 to 2 grams. Checks were run by inoculating plants with the juice of healthy tobacco plants and with sterile tap water. Ten plants were used in each case. No infections whatever occurred, and this is particularly significant since all extracts gave a strong oxidase reaction with guaiacum and hydrogen peroxide.

It would therefore seem that during the time which elapsed between the chilling of the plant and making the inoculations, the metabolism of the plant had been altered sufficiently to destroy the infective principle which the shoots originally contained. One can readily conceive of such an alteration, if we accept the enzymic theory as an explanation of the cause of the disease. The mottled shoots which had remained on the old stalk lost the sharp definition between lighter and darker areas, and the green shaded gradually into the lighter areas, the general position of which was marked by a yellowish brown spot. These results also show that the infective substance is not found in the tissue of normal plants.

Recovery from mosaic has been denied by many workers, and we are not in a position at this time to state that the experiments reported above demonstrate actual recovery. The failure of most workers to observe anything in the direction of recovery of diseased plants may probably be accounted for by the fact that the work on this phase of the problem has been rather limited. Some work in this direction has been done by liming the soil, etc., but no elaborate experiments have been performed. The work has furthermore been done under environmental conditions which favored the development of the disease. We may rest assured that when plants are grown under such conditions that the spontaneous appearance of the disease is favorable, recovery from the malady will be exceedingly rare. This applies particularly to greenhouse

conditions. Plants grown on plots or in the field mature and die or are cut down or killed by frost, which also precludes the possibility of observing total or apparent recovery.

Relation of Light to the Mosaic Disease

The effect of intensity of illumination or shading upon the development of the disease has been studied by Westerdijk ('10), Sturgis ('00), Chapman ('13), and others. A determination of the effect of colored light upon the mosaic disease of tobacco was attempted in the work of Lodewijks ('10) and more recently in the experiments of Chapman ('16). The work of Lodewijks, repeated by Chapman, is in brief as follows:

It was desired to note the effect of red and blue light upon the development of the mosaic disease. The tops of badly mottled plants were therefore covered with hoods made of red and blue cloth which were allowed to remain for about 30 days. At the end of this period it was noticed that shoots under red hoods were somewhat less mottled, while those under blue hoods showed little or no evidence of the disease.

It is very evident, however, that a red or a blue cloth hood does not give us a red or blue light, and all that can be expected in these cases is a difference in the shading effect of these hoods. In order to determine the effect of different light waves, it would be necessary to grow the plants under glass as nearly monochromatic as possible. The results reported by Lodewijks and Chapman are entirely in accord with what one would expect if plants were shaded. The red hood would shade the plants to a certain extent, growth and metabolic activity would be less than normal, and the disease would be less pronounced. The blue hood, however, would absorb more light than the red hood, the shading effect would therefore be greater, and the mottling would be reduced correspondingly.

The effect of light on the development of the disease is no meager problem. There are at least two distinct phases. It is a well-known fact that the mere absorption of light by

plants will raise their temperature from 4 to 11° C. above that of the surrounding atmosphere. The effect of light as regards increase in temperature is therefore important. Still more significant is the influence of light on the course of certain chemical reactions. Photosynthesis, for example, does not proceed in the absence of light. This, however, is an extremely complex and incompletely understood example, and the following illustration may serve to make the point clearer. When two volumes of chlorine are mixed with one volume of methane and the mixture exposed to strong sunlight, a violent explosion occurs, resulting in the formation of hydrochloric acid and the deposition of carbon, i. e., $CH_4 + 2Cl_2 = C + 4HCl$. If the mixture is kept in the dark or diffuse light, chlorine substitution products are formed, i. e., $CH_4 + Cl_2 = CH_3Cl + HCl$, $CH_3Cl + Cl_2 = CH_2Cl_2 + HCl$, etc. This simple experiment serves to show the importance of light stimulus in the determination of the direction of certain chemical reactions. Since light plays such an important rôle in the metabolic activities of green plants, its effect upon the manifestation of such a disturbance as the mosaic disease must be interpreted with the greatest reservation.

TRANSMISSION OF THE MOSAIC DISEASE THROUGH THE SEED

A great deal of dissension may be noted in the literature on the transmissibility of the mosaic disease through seed. The early appearance of mosaic, which may occur in the second leaf, has led certain workers to conclude that the disease must be carried over in the seed; yet, another sowing from the same sample of seed may yield plants which do not become diseased until they are half grown. Still greater confusion results when the idea is advanced that injury in transplanting predisposes the plants to the malady.

An attempt was made to throw further light upon this question, the method of growing plants in paper boxes as described above being adopted. About 1500 plants were handled in this manner. In some instances the tobacco seed was first sown in flats and then transplanted, while in other cases the

seed was planted directly in the boxes. No consistent results warranting a definite decision with respect to the transmission of the disease through the seed was obtained. Neither does it seem possible by the method employed that injury can be a very great factor, and it should therefore be regarded as incidental or disregarded entirely.

It has been the general experience of all workers that the seeds of diseased plants usually give rise to healthy plants, and this in itself is evidence strongly in favor of the non-transmissibility of the disease through seed. If we consider this question from the standpoint of an organism or parasite, one can only arrive at a satisfactory explanation by assuming that the virus present in the placenta (Allard, '15) cannot penetrate the integuments of the ovule and is thus filtered out. A much better explanation is afforded by the physiological aspect of the problem. In this case we should not expect to find the infective principle present in the seed. Mosaic is most pronounced in young shoots where growth, photosynthetic and metabolic activity is at its height. It is in these tissues that plant products are first formed. In the seed the physiological functions are entirely different. The relatively simple compounds elaborated in the green portion of the plant are polymerized there into storage products and no initial synthesis whatever occurs. The infective principle, if elaborated in young active shoots, may be transmitted to all parts of the plant through the general food stream and might therefore be present in the placenta. It might even enter the ovule, but on account of the specific functions of the ovule, embryo, and endosperm, the infective principle or corresponding enzyme might be altered and its continued formation obviated. We must bear in mind that in a problem of this kind we are dealing, from the standpoint of the host, with an extremely complex organism and must not confuse an intricate, nevertheless complete, chemical reaction or function exhibited by it with such a phenomenon as, for example, multiplication of bacteria or ultramicroscopic parasites. This will be discussed again later on.

THE INFECTIVE PRINCIPLE REGARDED AS AN ORGANISM

The work reported above was undertaken with the hope of obtaining information on the chemical or physiological nature of mosaic diseases. The problem was not, however, treated solely from this standpoint, but was undertaken in an unbiased attitude with the hope of gaining any possible information on all sides of the problem. A set of experiments was therefore planned which might bring out more clearly any relation of mosaic diseases to parasites or filterable organisms.

One method of attack was that of growing plants under sterile conditions. If plants grown under sterile conditions contracted the disease, the cultures otherwise remaining clean, one would, from a uniformity of results, be justified in concluding first, that the disease originated within the plant and that it is really of a metabolic or physiological nature, and second, that we have actually encompassed all physiological factors necessary for its production. If, on the other hand, no mosaic occurred, one would nevertheless not yet be justified in concluding that the disease must be due to an organism, since we are in the first place absolutely ignorant of the cause of the disease, and the physiological factors necessary for its production may be absent or subdued.

The cultures were prepared in the following manner: Jars (measuring 20 × 8 cm. and 16 × 10 cm.), specimen tubes (40 × 5 cm.), cylinders (48 × 5.5 cm. and 36 × 8 cm.), and tall specimen jars (varying in size from 40 × 11 cm. to about 60 × 16 cm.) were used. A certain amount of soil, the quantity varying with the size of the jar, was placed in each jar and a proportionate amount of water was added. All vessels, with the exception of the tall specimen jars, were sterilized for 4 hours at 15 pounds pressure. The latter were sterilized with formaldehyde, rinsed with sterile distilled water, and into them a certain amount of sterile soil was then placed. Soils from different sources were used, i. e., from the plot containing diseased plants and from greenhouse plots in which diseased material had been grown. Soils from the to-

bacco and cucumber plots were kept separately. The seed was treated in different ways. The commercial seed was sterilized with formaldehyde and a check was run against this with unsterilized seed. Seed was then collected from diseased tobacco and cucumber plants and applied in a sterile and non-sterile form to sterile and non-sterile vessels.

The necessity of growing plants which are to be used in physiological experiments under sterile conditions, has been recognized for some time, but comparatively little stress has been laid upon the desirability of employing sterile cultures of host plants in pathological work. When dealing with problems such as are encountered in the "physiological" diseases, methods like the above become quite essential. In order that contamination might be detected in the sterile cultures, about 5 to 10 cc. of ordinary potato agar was poured on top of the soil before planting the seed. The number of cultures totaled 352. The cultures were set up October 29 and 30, 1916, and allowed to run until January 10, 1917. At this time many of the plants had died, none of them, however, having shown the slightest indication of mosaic. The negative results obtained do not prove nor disprove anything, and this is particularly true, since the spontaneous occurrence of the disease was not observed anywhere in the greenhouse at that time of the year. The experiment will be repeated as soon as time permits.

Another line of attack on this phase of the problem was the detection of metabolic activity in a medium containing an extract of diseased plants. The technique involved, however, was more complicated than was originally supposed, and while no results are available at this time, it is hoped that the experiments outlined will prove of great value. If the infective principle is of the nature of an enzyme, we should expect a definite chemical reaction to occur which should be governed by the laws dominating chemical reactions. The speed of the reaction might be influenced by acidity or alkalinity, but there should be no change in the nature of the end product. If, on the other hand, the infective principle is of the nature of an organism, we should expect a relatively

simple constituent of the medium to be destroyed or used up in the metabolism of the organism. The organism should furthermore not be restricted to the use of a single compound as a source of food, but substitutions could be made. The metabolism would furthermore not be governed to the same extent nor in the same manner as are single chemical reactions, though it would undoubtedly be influenced by physiological relations much the same as are other organisms.

NEW AND RECENTLY DESCRIBED MOSAIC DISEASES

Although cucumber mosaic has been known for several years, its economic importance has not been appreciated until recently. Since the prevalence of the disease is only partially known, it is difficult to estimate the annual loss involved, but it undoubtedly approximates a million dollars annually. It is reported from an area bounded by Minnesota, Colorado, Mississippi, Virginia, and New York. The foliage of diseased plants always assumes the curled or crinkled and mottled appearance characteristic of mosaic diseases, while the fruit may remain small and cone-shaped, or become extremely "warted" and mottled, or only mottled and not greatly deformed as shown in pl. 16, fig. 1.

The new mosaic disease of peanuts has recently been described by McClintock ('17). But a single plant was found, and all inoculation experiments gave negative results. On account of shortage of material with which to carry on extensive inoculation experiments, it was impossible to establish definitely the identity of the disease.

A disease appearing spontaneously on avocado plants[1] grown in the greenhouse for experimental purposes, showed all the characteristics of mosaic diseases. So far as can be ascertained by the writer, no observations of this kind have ever been made. The plants were grown in a room together with diseased tobacco plants, and it is therefore impossible to state whether the disease was contracted from the tobacco or whether it is distinctive of the avocado. The plants are

[1] The seeds for these plants were obtained from Santa Anna, California, through the courtesy of W. S. Reeves.

now being used in connection with physiological experiments, and want of material has precluded carrying on inoculation experiments, though this will be done as soon as possible. Plate 17 shows the healthy and diseased avocado plants.

Discussion of Recent Investigations

Whether or not mosaic diseases are initially "physiological" or are caused by an organism, it is nevertheless apparent from the preliminary results on physiological relations reported above that the physiological side of the problem is an extremely important one. After the physiological relations are more clearly understood, we will undoubtedly be able to account more fully for some of the inconsistencies of results, such as failure to get infection after inoculation or the spontaneous occurrence of diseased plants among checks. This is true regardless of the origin of the disease. Since so little is known concerning "physiological" diseases, it is impossible to cite conclusive data as regards environmental influences exerted on their causes.

In the case of diseases which have been proven to be due to well-described organisms, we are able to correlate the effects of environmental factors with something tangible and the results are always obvious. In any event, such problems must be considered from two different aspects, i. e., from the standpoint of the host and from the standpoint of the parasite. Work on temperature and host relations, covering a period of several years, has recently been reported by Gilman ('16). Our present knowledge on the subject has been admirably summarized by him. The importance of physiological pathology has also been brought out by the work of Selby ('99), Orton ('13), Halsted ('98), Balls ('08), Reed ('10), Earle ('02), and others. If mosaic diseases are caused by an organism, the effect of those climatic conditions favorable for their manifestation may be accounted for, first, by the favorable effect exerted upon the organism, and second, by the favorable effect on the host which, however, makes it better prey for the parasite. If, on the other hand, mosaic diseases

are "physiological" in origin, then any physical condition which would accelerate a particular type of chemical reaction would tend to make the disease more pronounced.

It was not until July, 1916, that any experiments were reported on the properties of the infective principle of mosaic diseases. Allard ('16[a]) at this time reported on a set of experiments which he interpreted as further evidence in favor of his view that mosaic diseases are due to a filterable parasite or "virus." His results are in brief as follows:

A number of filtration experiments were carried on, from the results of which Allard concluded that the organism was filtered out. These results can, however, be explained on an entirely different basis. Lacking a Berkefeld filter, he filtered the extract through a Livingston atmometer porous cup. It was found that the resulting filtrate contained no infectious substance. Although one might conclude that if an organism had been present, it was filtered off, it nevertheless does not preclude the possibility that a colloidal compound or enzyme, because of its relatively large particles or partial absorptive phenomena, might not also have been arrested by the filter. The extract was next filtered through powdered talc. It was found that if a certain amount of extract was filtered through a certain amount of talc, a stage was reached at which all of the infectious properties were filtered off. However, on studying the data, we notice that the oxidase activity was also destroyed entirely or reduced correspondingly. This should therefore not be interpreted as a simple filtration experiment with an organism, but as an illustration of the high absorptive properties possessed by colloids in general and therefore by enzymes, such as the oxidases and probably the infective principle of mosaic diseases.

Precipitation experiments with ethyl alcohol were also carried out by Allard. For this purpose 45, 50, 75, and 80 per cent alcohols were used. In each case a certain amount of extract was taken and enough absolute alcohol added to give the desired concentration. The mixture was allowed to stand from 1 to 2 days, at the end of which time the precipitate was filtered off and dried at room temperature. Suspensions of

this were then used for inoculations. The precipitates obtained with 45 and 50 per cent alcohol produced the disease, while those obtained with 75 and 80 per cent alcohol gave negative results. Any one familiar with the preparation of enzymes is conscious of the fact that the higher concentrations of alcohol destroy most enzymes in a comparatively short time, and this is probably what happened when Allard treated his material with alcohol for 2 days. Dilute concentrations of alcohol do not exert a deleterious action, and 20 to 30 per cent is therefore often used during the process of extraction in order to prevent bacterial action. The results of Allard should, therefore, not be interpreted as proof of the destruction of the mosaic organism by higher concentrations of alcohol, but rather to illustrate the deleterious effect which high concentrations of alcohol exert on enzymes such as the infective principle of mosaic diseases.

Extracts were next treated with hydrogen peroxide in order to destroy the oxidases. A concentration was found at which all oxidases were destroyed, but the infective principle was retained. It was this method which enabled Allard to demonstrate that mosaic diseases are not caused by oxidases. The problem was then attacked from the other angle and the infective principle was destroyed while the oxidases were retained. This was accomplished by adding different concentrations of formaldehyde. When concentrations of formaldehyde of 1:800 and 1:1000 were employed, only 1 plant out of 10 became infected. When greater concentrations were used no infections resulted, while with greater dilutions the infectious properties were retained to a considerable degree. Although not specifically stated by Allard, this was presumably interpreted to mean that formaldehyde was penetrating enough to kill the organism. On the other hand, it suggests a specificity of reaction of a compound with formaldehyde, and probably with aldehydes in general. Furthermore, if formaldehyde is one of the first products of photosynthesis, as contended by Usher and Priestley ('06, '06[a]), Schryver ('10), and others, one can easily conceive of a physiological origin of mosaic diseases. There is less carbohydrate in the

lighter areas of diseased leaves than in the darker. If the metabolism of the cells of the lighter areas is such as to arrest the formation of formaldehyde, the formation of unusual enzymes might take place which, when introduced into a normal healthy individual, are capable of reproducing themselves and stimulating a pathological condition in a manner which will be described later.

Dried and ground mosaic material was next treated with various organic extractives. Ten grams of dried material were treated with 70 cc. of the extractive for 2 days. At the end of this time the extractive was filtered off and evaporated at room temperature. Inoculations were later made with water suspensions of the residue obtained after the evaporation of the extractive, and also with water extracts of the material which had previously been treated with the extractives. The extractives used were ether, chloroform, carbon tetrachloride, toluene, acetone, ethyl alcohol, methyl alcohol, and glycerin. No infections (with one exception) resulted when inoculations were made with the water suspension of the residue obtained after the evaporation of the extractive. This exception was that of glycerin. In this case, however, the residue had been macerated with the extractive, and Allard later found that if the glycerin was simply allowed to act on the dried mosaic material and was then poured off, it contained little, if any, of the infectious principle. When inoculations were made with the water extract obtained from material which had been previously treated with various extractives, infections resulted in all cases except in those where alcohol had been used.

All this is entirely in accord with what one would expect if we were dealing with an enzyme. If the infectious substances were of the nature of an organism, it certainly should have been destroyed by treatment for 2 days with concentrated solutions of such antiseptics as ether, chloroform, carbon tetrachloride, acetone, toluene, and glycerin. It also lends further proof to the contention that in the case of treatment with formaldehyde the destruction of the infectious substance was due, not to the antiseptic properties of formaldehyde, but

was the result of a chemical reaction; and the reason for the destruction of the infective principle at a concentration of 1:800 is that it required this quantity of formaldehyde to balance the reaction. Glycerin, in varying concentrations, is frequently used as an extractive for enzymes. The solution of the enzyme actually takes place in the water, but the use of glycerin is advantageous on account of its penetrating power and preservative properties.

The other extractives employed by Allard, when used in connection with enzyme work, are used only as preservatives. The fact that the infective principle can be extracted only with water is in accordance with the common practice of securing enzymes by dissolving them in water or obtaining them as aqueous extracts. The action of the alcohol on the dried material was two-fold. In the first place, it had a tendency to precipitate the enzyme in the tissue, thus making subsequent extraction impossible, and in the second place, the alcohol exerted a destructive influence upon the enzyme.

Allard also treated green tissue with extractives. In these cases he was able to extract the infective principle at least to some degree. However, this should be regarded as a solution of the enzyme in the water naturally present in the plant, the extractives merely acting as antiseptics.

It was also found that the "virus" could be thrown out of suspension with precipitates of aluminium hydroxide and nickel hydroxide. This merely demonstrates the familiar process of flocking out colloidal suspensions and is entirely applicable to enzyme solutions.

The effect of heat was tested on both wet and dry material. Extracts, according to the results of early workers, lose their infectious properties at 65–75° C. Although this is the lethal temperature for many organisms, it is also the temperature at which most enzymes are deactivated. Allard states that "the infective principle of the virus is quickly and permanently destroyed at temperatures near the boiling point. . . ." This is not only applicable to enzymes but also to other compounds readily undergoing hydrolysis. Dried heat destroyed the infective principle of dry material at 130° C., but Allard's

data show that oxidase activity was also destroyed at this point. A temperature of —180° C. did not destroy the "virus." Although some organisms can withstand a temperature as low as this, it is also a fact that chemical compounds, including enzymes, can be cooled to any degree without changing their constitution.

Various workers have found that fermented extracts of mosaic material gradually lose their infectious properties. This has also been experienced by the writer. Allard ('16[a]), on the other hand, states "that the virus will retain its infectious properties almost indefinitely without the addition of toluene. With no preservative whatever added, the bottled virus was highly infectious when tested 12 to 15 months later, although putrefaction had taken place." We are not in a position to discuss this matter at this time since we are absolutely ignorant of the cause of this putrefaction. It might have been due to the action of bacteria, of wild yeasts or fungi, or the activity of autolytic enzymes present in the extract. On the other hand, if the infective principle is as sensitive to formaldehyde as Allard's results indicate, the destruction of the infectious properties might have been due to the formaldehyde resulting from the oxidative decomposition of chlorophyll (Warner, '14). The extracts are preserved as aqueous solution, generally using toluene as a preservative. This is exactly the manner in which enzyme digestion mixtures are set up, which is further indication that when putting up an extract of mosaic material in this manner, we are preserving an enzyme and not an organism.

Considerable disagreement may be noticed in the literature as regards the transmissibility of the mosaic disease of certain plants to other species. Some of the work on this phase of the problem is reviewed briefly in a recent article by Allard ('16[b]), and results are reported which indicate that the mosaic disease of *Nicotiana viscosum* is distinct from that of *Nicotiana tabacum*. These results might lead one to conclude that these are "biological species" or "physiological races" of the mosaic "virus." However, it is a well-known fact, particularly in animal physiology, that fluids,

toxins, or enzymes from one species may not affect closely related species. The constitutional or physiological differences between two species make these organisms two different species, and the ability of certain plants to resist the influence of certain mosaic extracts can be accounted for on physiological grounds. It is the physiological difference between hosts which makes some of them immune to a disease while others are susceptible, and it is likewise a physiological difference among parasites which makes "physiological races" *physiological* races. The term is used primarily in connection with the parasitism of rusts, mildews, etc., which may be detected with the naked eye and are adequately described. The term should not be used in connection with the infective principle of mosaic diseases which has never been seen, never been described, and the "properties" of which are only partially known. If the term is used at all it should be used only as a matter of convenience, and this is discouraged since it tends to lead to confusion.

From the above it is obvious that when injecting the infective substance obtained from a diseased plant into a healthy plant, we are handling an enzyme and not an organism. Although nothing is known as regards the nature of this enzyme, it is probably, judging from its reaction with formaldehyde, of the nature of an aldehydase. If formaldehyde is one of the first products of photosynthesis, one can easily conceive of a physiological origin of mosaic diseases. A probable relation with photosynthesis is furthermore brought out by the observation on the carbohydrate content of the lighter and darker areas of diseased leaves, as was pointed out in the microchemical work reported above. The problem has, in the light of these facts, assumed a somewhat different aspect. Although nothing is known as to the nature of the enzyme, the main issue of the problem is this: How does this enzyme originate and what are the factors which induce its formation? As was pointed out by the writer earlier in this paper, and as has also been shown by Allard, this infective principle is not found, at least in active form, in healthy plants grown under normal conditions. If it is

present, inhibitory factors are also present which hold it in check, determine its reactions, and do not allow it to be formed to such an extent as to exert pathological influences. It is of course true that we have no basis for assuming that this supposed enzyme is the initial cause of the disease, and may be considered by some as a result of the disorder, but we are nevertheless forced to the conclusion that when this supposed enzyme is injected in an active form into healthy plants, it is capable of stimulating its further production, and therefore we have reason to believe that it is the causal agent in all cases. The physiological conditions which determine its production form the nucleus of another problem.

A point which may seem to be greatly in favor of the "virus" theory is that the extract may be diluted 1:1000 or even 1:10,000 and still retain the capacity of inducing the disease in healthy plants. It is a well-known fact, however, that nearly all chemical reactions reach their termination better and more completely when the chemicals are brought together in *relatively* dilute concentrations. This is particularly applicable to substances of a colloidal nature. Chemical reactions resulting from the activity of such colloidal compounds as enzymes, are largely dependent upon the adsorptive power of these enzymes. If, then, they are present in relatively dilute concentrations, the colloidal particles will be more dispersed, the opportunity for adsorptive phenomena greater, and chemical action free to proceed in its normal course. If the enzyme producing mosaic diseases is extremely active, one may easily understand how great dilution would yet enable it to induce metabolic disturbances. When reactions of this kind are carried on *in vitro,* the activity, on account of a limited amount of material, will ultimately cease. In a living organism, however, the situation is entirely different. More compounds are constantly being formed as the result of metabolic activity. When these compounds are acted upon by the enzymes, the end products or the intermediate products formed may stimulate the formation of more of the enzyme, which in turn will lead to further disturbances.

This may be hard to understand, but that similar phenomena do occur is an established fact. It has been demonstrated in animal physiology as well as in plant physiology. Abderhalden and his co-workers have found that if, for example, native protein, proteoses, or peptones are introduced parentally into an organism, enzymes normally not present in the blood will be formed (Underhill, '15). The proteolytic enzymes produced hydrolyze the compounds to amino acids which are then absorbed from the blood stream by the tissues. When hydrolysis of the compounds has been completed, the enzymes disappear again, but will be reformed upon the injection of more of the proteinaceous substances. Knudson ('13) found that tannase is not produced by *Aspergillus niger* nor by certain species of *Penicillium* if tannic acid or its decomposition product, gallic acid, is omitted from the nutrient solution. The amount of tannase produced increases in accordance with the concentration of the acids. Many other examples might be cited, all of them illustrating the same point.

If the mosaic enzyme acts upon a compound present in the healthy plant, or if in the process of photosynthesis it determines the formation of certain compounds, we can easily conceive how the presence of some of the end products or intermediate products may stimulate the formation of more of the enzyme. We must remember that after the enzyme has once been introduced into the plant, it plays a part in, and in fact becomes a part of, the metabolism of the plant. This fact becomes obvious when we consider the malformations and the large amount of "infective principle" that the substance gives rise to when injected into normal plants.

These interpretations are entirely in accord with the fundamental principles upon which all our scientific conceptions in pathology and biology are based. The continued formation of the mosaic enzyme when once introduced into a healthy plant has been accounted for on purely physiological grounds. It is of course true that self-reproduction is a characteristic of living things, but this must not be confused with the reproduction of chemical compounds, including enzymes, in a highly

developed and complex organism. The ability to produce these compounds according to the needs of the organism has been demonstrated by the work of Abderhalden and his collaborators, by Knudson, and others. We cannot compare the functions of a complex organism, such as the ability to produce certain compounds in accordance with its need for them, or the ability to determine the course of extremely complex, yet complete, chemical reactions, with a function which the organism can perform only as an entity, such as self-reproduction. We furthermore must not confuse "self-reproduction" in comparatively simple organisms, like the bacteria, with the production and reproduction of enzymes in the higher plants and animals. It is likewise true that many infectious diseases are associated with parasitism, but there are many which have not found an explanation in this cause. Examples of this in animals are measles, chicken-pox, mumps, scarlet fever, etc., while the group of "physiological diseases" of plants serves as an example for the vegetable kingdom.

The fact that self-reproduction in a simple organism and the production of certain substances in a complex organism are two entirely different things is furthermore demonstrated by the following example. We are all familiar with the fact that the pathological condition characterizing diphtheria is attributable to the toxin produced by *Bacillus diphtheriae* which has lodged itself in the pharyngeal passages. If a portion of the toxin is injected into a normal individual, he will succumb to the pathological condition, i.e., lesions of the heart, nerves, kidneys, etc., characteristic of diphtheria, and additional toxin will be produced in his system; yet no organism has entered into the case. The inflamed condition of the throat will, of course, be absent, but this is largely the result of local irritation. Similar reactions occur in the production of serums, anti-bodies, and the like in other diseases, and it is upon the ability of an organism to produce and reproduce such complex substances and enzymes that the science of immunology is based.

It is unfortunate that we have to go to the field of animal pathology for examples of this sort, but we are forced to do

so because of the complexity of these reactions and, furthermore, because of our ignorance of such things in the vegetable kingdom. The writer does not wish it to be thought that in drawing upon animal pathology for examples, an opportunity is sought for begging the question of the production of the mosaic enzyme in infected plants. It merely serves to illustrate the fact that the production of the mosaic enzyme is no more complex than the production of toxins, serums, and the like in animal pathology, all of which are accounted for on physiological grounds.

In the light of all evidence now at hand, we must consider the infective principle of mosaic diseases as being an enzyme, and in doing so we do not abuse any of our fundamental biological conceptions of pathology and physiology.

Summary

The evidence that has accumulated from the efforts of recent workers on mosaic diseases and that presented in this paper enable us to formulate the following summary:

1. Mosaic diseases are not caused by an unbalanced inorganic nutrition. The inorganic elements are present in diseased and healthy tissue in relatively the same amounts.

2. Carbohydrates are more abundant in the dark green than in the light green areas, regardless of the time of day.

3. Proteins are present in both the lighter and darker areas. Preliminary nitrogen analyses indicate that the quantity of protein in the lighter areas is slightly in excess of that in the darker areas.

4. Whether or not the disease is initially due to physiological disturbances or to parasites, the physiological phase is an extremely important one.

5. Preliminary observations on temperature relations indicate that there is not only an optimum for the manifestation of the disease, but also a maximum and minimum above and below which the disease is checked. The

development of the disease is arrested sufficiently to suggest apparent recovery.

6. Properties of the infective principle substantiate the view that the infectious substance is an enzyme and not a "virus." This enzyme is not of the nature of the oxidases giving the guaiacum reaction.

7. The infective principle is greatly adsorbed by talc, a phenomenon characteristic of all colloidal compounds including enzymes.

8. There is a specificity of reaction between the infective principle or mosaic enzyme and formaldehyde and probably with aldehydes in general.

9. The destruction of the infective principle cannot be attributed to the antiseptic properties of formaldehyde, since treatment with concentrated solutions of the best antiseptics as ether, chloroform, carbon tetrachloride, toluene, acetone, and glycerin does not destroy the infectious properties.

10. The infectious properties are destroyed by concentrations of alcohol which are destructive to enzymes.

11. The temperatures which destroy the infectious properties are the same as those which inactive enzymes or hydrolyze some organic compounds. Cooling has no greater effect on such properties than is exerted on any chemical compound, including enzymes.

12. The reproduction of the mosaic enzyme can be accounted for on purely physiological grounds, but the factors which originally induced its formation are still unknown.

13. The specificity of reaction of the mosaic enzyme with formaldehyde and the unbalanced carbohydrate relation between lighter and darker areas, combined with the contention that formaldehyde is one of the first products of photosynthesis, suggest a basis upon which the physiological nature of mosaic diseases may be explained.

14. The continued production of the mosaic enzyme in inoculated plants is in accord with the fundamental principles of pathology and physiology.

In conclusion the writer wishes to express his indebtedness to Dr. G. T. Moore and the Missouri Botanical Garden for generously providing facilities with which to prosecute the problem, and also, to thank Dr. B. M. Duggar for numerous suggestions and helpful criticisms.

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EXPLANATION OF PLATE

PLATE 14

Fig. 1. Effect of low temperature (45° F.) upon the development of the mosaic disease of tobacco: *a*, old leaf still showing mosaic; *b*, young leaf showing very slight venation at the tip, but otherwise normal; *c*, older leaf showing slight venation, but no mottling.

Fig. 2. Effect of high temperature (85° F.) upon the development of the mosaic disease of tobacco: *a*, young leaf showing venation; *b*, older leaf showing venation changing to mottling; *c*, old leaf showing mottling. This leaf is about the same age as leaf *c* in fig. 1.

Fig. 3. Showing change from venation to mottling, temperature 85° F.: *a*, some venation still present in tip of leaf, but otherwise mottled; *b*, younger leaf venated throughout.

1

2

3

FREIBERG—MOSAIC DISEASES

EXPLANATION OF PLATE

PLATE 15

Fig. 1. Effect of temperature on the development of the mosaic disease of tobacco. Plants *b* and *c*, grown at a temperature of 65–75° F., are the same age. Plant *b* is apparently affected with mosaic, while *c* is perfectly normal; photographed November 24, 1916. Plant *a* shows *b* on December 13, 1916, after having been kept at a temperature of about 40° F. and illustrates apparent recovery.

Fig. 2. An old diseased plant transferred to the greenhouse in the fall. Shoots *a* and *b* appeared while the temperature was low (about 40–45° F.) and illumination poor, and do not show mottling. Shoot *c* appeared while the temperature was high (about 60–70° F.) and sunlight more abundant. Slight mottling is evident.

FREIBERG—MOSAIC DISEASES

EXPLANATION OF PLATE

PLATE 16

Fig. 1. Mosaic disease of cucumber.

Fig. 2. Mosaic disease of tobacco.

Fig. 3. Mosaic disease of citron.

1

2

3

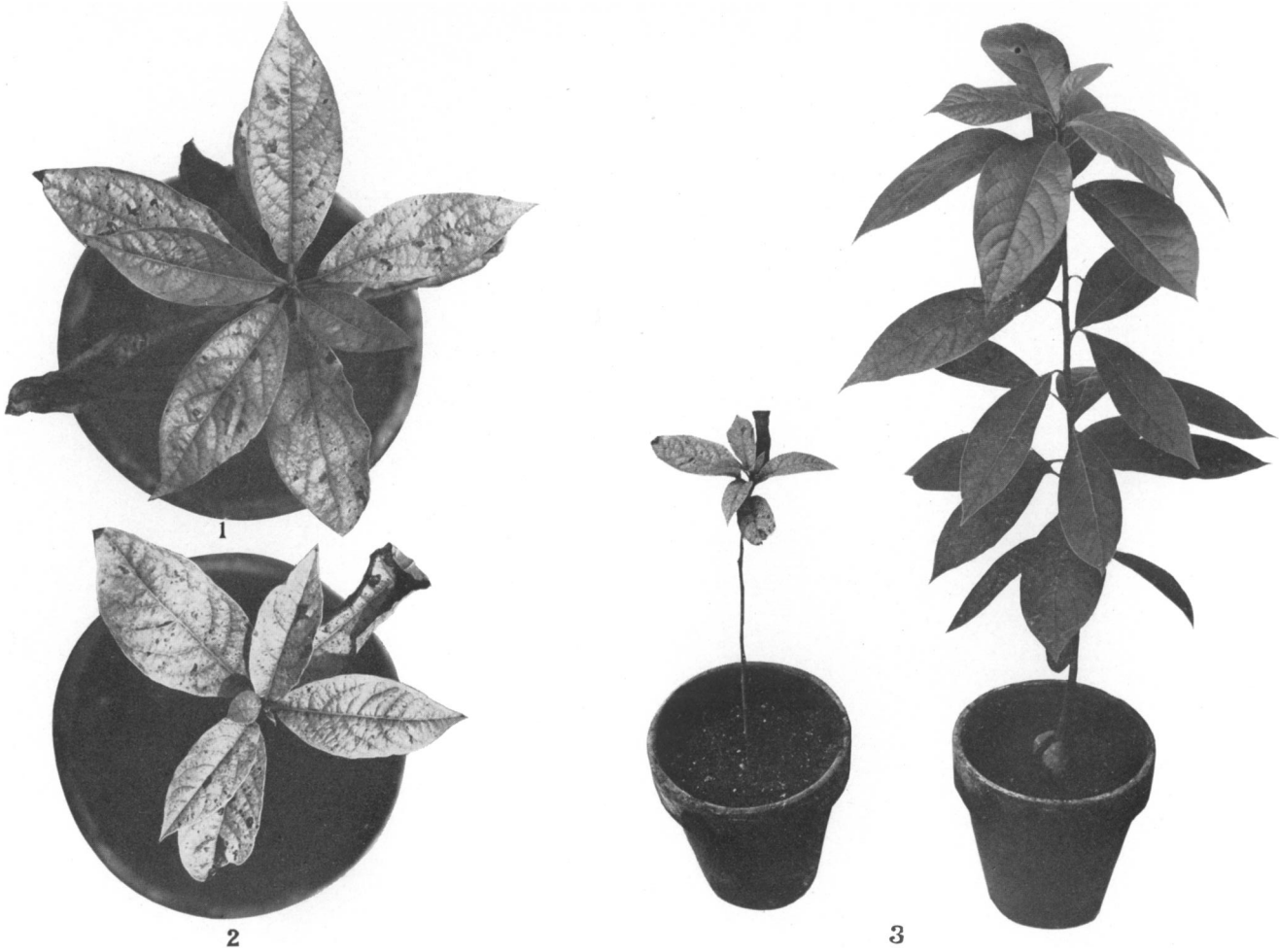
FREIBERG—MOSAIC DISEASES

EXPLANATION OF PLATE

PLATE 17

Figs. 1 and 2. Mosaic disease of avocado.

Fig. 3. Diseased and healthy avocado plants; seed planted November 1, 1916; photographed March 28, 1917.



FREIBERG—MOSAIC DISEASES